INHERITANCE OF RESISTANCE TO TOMATO SPOTTED WILT VIRUS

IN LETTUCE (LACTUCA SATIVA L.)

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ABSTRACT

Tomato Spotted Wilt Virus (TSWV), a thrips transmitted virus disease with a wide host range, has become a major limiting factor in lettuce production in Hawaii. Symptoms of this disease on lettuce are necrotic spotting of young leaves, stunting, wilting, and death of the plant. The large host range of this disease has made it difficult to control. Genetic resistance might work, but there have been no reports of resistance in lettuce.

Hartmann (personal communication) undertook a preliminary screening program for TSWV resistance and found six lines of lettuce 'Tinto', PI 167128, PI 342510, PI 342517, PI 342522, and PI 342526 that showed less infection with TSWV than the susceptible 'Manoa'.

In this study the six lines that Hartmann found plus one more 'Batavia' were tested for TSWV resistance. Only two lines, 'Tinto' and PI 342517 showed significantly higher resistance than the susceptible 'Manoa'. These two resistant lines were crossed with 'Manoa' and each other to produce F_1 and F_2 seed. Results of testing the F_1 and F_2 plants suggest that 'Tinto' and PI 342517 have the same genes for resistance and that this resistance is controlled by a dominant or partially dominant gene complex.

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INTRODUCTION

Lettuce, *Lactuca sativa* L. is a major vegetable throughout the world. In the United States, lettuce is the leading fresh market vegetable crop in acreage, production, and market value (Ryder 1986). In Hawaii in 1985, lettuce held a similar status of importance among vegetable crops by ranking first in acreage with 800 acres, second in production at nearly 12 million pounds, and third in market value at nearly 2.9 million dollars (Statistics of Hawaiian Agricultural 1985).

Three types of lettuce are predominately grown in Hawaii: heading, often referred to as crisphead or iceberg, semi-heading, sometimes known as butterhead, and Romaine or cos. All three types of lettuce are highly susceptible to attack by the disease Tomato Spotted Wilt Virus (TSWV). This virus disease has become a major limiting factor to lettuce production in Hawaii. In several areas lettuce production has been reduced or discontinued entirely because of TSWV. Common symptoms of TSWV on lettuce include brown necrotic spotting of young leaves, stunting of growth, and wilting and death of the plant.

This destructive virus has a wide host range including the tomato, *Lycopersicon esculentum* M.. In the tomato, resistance to TSWV has been located and its inheritance system has been identified (Finlay 1953). This knowledge has been put to use by plant breeders in developing TSWV resistant tomato varieties.

Until recently, TSWV on lettuce hasn't been a persistent problem and thus no inheritance studies on resistance have been reported. However, with the continuous problem of TSWV on lettuce in Hawaii, Hartmann (personal communication) undertook a preliminary screening program for TSWV resistance. He was able to find several lines of lettuce that expressed some resistance to TSWV under certain environmental conditions. The purpose of this study is to confirm the existence of this resistance where present, determine whether it is genetically controlled, and if so, investigate its inheritance.

LITERATURE REVIEW

Botany and origin of lettuce

Lettuce most likely originated in the Mediterranean basin. Even today a large diversity of lettuce types can be found in this area. The earliest recorded evidence of lettuce is from the tomb paintings in Egypt, which date back to about 4500 B.C.. The plants depicted in the paintings are narrow leaved, perhaps indicating an early form of cultivated cos lettuce (Ryder, 1979).

Ryder (1986) speculates that lettuce originated along the Mediterranean sea coast, most likely in Egypt, and then spread to the interior. This spread of lettuce from the mild temperatures of the coast to the interior regions brought about evolutionary changes. Warmer areas evolved slower bolting forms which maximized leaf development and competitive ability, while colder sections evolved long-day flowering types that insured reproduction.

Lettuce is in the Cichoreae tribe of the Compositae family. Bailey (1976) describes it botanically in the following manner. "Annual or biennial, to 3 feet; leaves in a basal rosette before flowering, to 10 inches long, entire or runcinate-pinnatifid, stem leaves ovate to orbicular, entire, glabrous, sessile; heads less than 1/2 inch across, many in a dense corymbose panicle; flowers pale yellow; achenes straw-colored or black, with white beak".

Cytogenetics of lettuce

L. sativa is a diploid with the chromosome description of 2n = 2x = 18. The genus *Lactuca* has about 300 species, all of which are highly self pollinated. *L. serriola*, *L. virosa*, and *L. saligna* along with *L. sativa* form a breeding group in which all four species have 18 chromosomes. *L. serriola* crosses easily with *L. sativa* suggesting that these two species should be subsections of the same species. The other members of this group don't cross as readily and often have hybrid sterility problems (Lindkvist, 1960).

Types of lettuce

There are six morphological types of cultivated lettuce: butterhead, cos, crisphead, Latin, leaf, and stem. Butterhead lettuce is also known as bibb or semi-heading. This type of lettuce has older leaves that fold over younger leaves to make a loose head with inner leaves lighter green in color than outer leaves. Cos lettuce, often referred to as romaine, is similar to butterhead in that both have relatively loose heads, but cos leaves are much coarser and elongated. Crisphead, the most important lettuce type in the United States, is known as head or iceberg lettuce. This lettuce is similar to butterhead and cos in that older leaves fold over younger leaves, but in crisphead the leaves fold much tighter. This results in inner leaves more succulent in nature, and much lighter, often white to yellowish in color. Latin lettuce resembles a cross between butterhead and cos lettuce. Leaf lettuce can be characterized by lack of head formation. Stem lettuce, as implied by the name, is grown for the thick stem it produces with leaves being inconsequential (Ryder, 1979).

The History of Tomato Spotted Wilt Virus

The initial reports on the disease "spotted wilt of tomato", later to be known as TSWV, were by Brittlebank (1919) and Osborn (1919). Brittlebank's report included a description of this new disease first observed on tomatoes in 1915 in the State of Victoria, Australia. Osborn reported seeing this same disease on tomatoes in South Australia. By the early 1920's, all states of Australia had incidence of this disease (Best 1968). Beginning with Smith's reports (1931, 1932) in the United Kingdom, in subsequent years reports of TSWV have appeared from the continents of Europe, Asia, Africa, South America, and North America (Best 1968). TSWV can thus be said to have a worldwide distribution.

Samuel et. al. (1930) working in Australia were the first to prove the disease organism is actually a virus and they characterized it as a single entity. However, Norris (1946) differentiated five strains, Best and Gallus (1953a) differentiated six strains, and Finlay (1953) listed 10 strains of the virus.

TSWV in Hawaii

Tomato Spotted Wilt Virus in Hawaii was initially known as yellow spot of pineapple. The description of this disease, which had been observed as early as 1926, was first published by Illingworth (1931). Linford (1932) proved this disease could be transmitted by the thrips species *Thrips tabaci*, but he was unable to conclusively prove the identity of the disease as tomato spotted wilt.

Parris (1938) reported an outbreak of a tomato virus disease on Oahu in 1937 which showed characteristics of spotted wilt virus. In a follow up report, Parris (1940) used mechanical inoculation to provide evidence that yellow spot and tomato spotted wilt virus are the same disease. Concurrently, the same conclusion was reached by Sakimura (1940) while he worked with the vector *Thrips tabaci*.

Throughout the late 1930's and early 1940's TSWV was a continuous problem for commercial tomato growers in Hawaii. In 1941 a breeding program was begun in Hawaii to develop tomatoes resistant to TSWV. After four years, the first commercial TSWV resistant tomato variety, Pearl Harbor, was released (Kikuta et al., 1945). However, this resistance didn't provide protection from TSWV in other areas of the world because it was strain specific for the strain of TSWV noted in Hawaii (Kikuta and Frazier, 1946). Consequently, the resistance of Pearl Harbor in Hawaii also broke down with the inevitable arrival of other strains (Upreti, 1983).

Oahu was the first island to have TSWV in epidemic proportions, and the leeward side of the island was most severely affected. By the late 1960's, the level of disease incidence had become so high it eventually resulted in the elimination of tomato production on the leeward side.

During this same time period several growers in this area were raising Manoa lettuce. Initially these growers encountered severe losses on their lettuce during the hot dry summers, but little damage during the rest of the year. However, by the 1970's, TSWV on lettuce in the leeward area had increased to such an extent, some growers discontinued lettuce production entirely (Mau, 1986).

Today TSWV continues to plague Hawaiian agriculture. Crops of head, semi-head, and romaine lettuce, tomatoes, and bell peppers are most affected. Losses during some seasons in lettuce fields can range from 50-90 percent . TSWV seems to be most prevalent on the islands of Oahu and Maui, and appears to be increasing on the island of Hawaii, especially in the Kamuela region (Mau, 1986).

Hosts of TSWV

Tomato Spotted Wilt Virus has an extensive host range including over 30 families and 200 species of plants (Best, 1968, Abu Bakar, 1974, Francki and Hatta, 1981, Cho et al., 1986). In Hawaii there are 44 plant species diagnosed as being naturally infected with TSWV. These plants act as the source of TSWV for the thrips vector (Cho et al., 1986). The major hosts of TSWV in Hawaii are listed in Table 1.

Duffus (1971), while examining the role of weeds in the incidence of virus, explains that an extensive host range enables a virus to have the potential for a larger distribution and the ability to be more persistent than a virus with a limited host range. TSWV in Hawaii can be classified as such a persistent virus.

Table 1. Important host plants of TSWV found in major vegetable growing regions of Hawaii (Cho et al., 1986).

Plant species	Common name
Amaranthus hybridus	---
Amaranthus spinosus	spiny amaranth
Amaranthus viridus	slender amaranth
Apium graveolens	celery
Arctium lappa	burdock
Bidens pilosa	spanish needle
Bidens pilosa var. minor	spanish needle
Capsella bursa-pastoris	shepard's purse
Chenopodium album	lambsquarters
Chenopodium murale	---
Coronopus didymus	swinecress
Ipomea congesta	blue morning glory
Lactuca sativa	lettuce
Leonotis nepetaefolia	lion's-ear
Lycopersicon esculentum	tomato
Malva parviflora	cheese weed
Melilotus officinalis	sweet yellow clover
Nicandra physalodes	apple of peru
Portulaca oleracea	purslane
Sonchus oleraceus	sowthistle
Stellaria media	chickweed
Tropaeolum majus	nasturium
Verbesina enceloides	golden crown's beard
Xanthium saccharatum	cocklebur

TSWV on lettuce

For over fifty years TSWV has been known to infect lettuce. Tompkins and Gardner (1934) noted heavy losses in head lettuce in coastal districts of California due to Spotted Wilt Virus. They also determined, by using reciprocal inoculation procedures between tomatoes and lettuce, that this was the same disease that attacked tomatoes. All the varieties they observed, including Romaine types, were susceptible to TSWV to the degree of either unmarketability or premature death.

Magee (1935) working in New South Wales reported 15-25% of the lettuce he observed were infected with TSWV. He also noted that unusually dry weather favored development of thrips, the vector of the virus. Like Tompkins and Gardner, he too observed that no varieties showed resistance.

Harris (1939) reported a major outbreak of TSWV on lettuce in California. However, since this 1939 report by Harris, TSWV on lettuce in California has not proven to be of major importance. The main indication that TSWV on lettuce was still present in California since 1939 is Borchers (1957). In his dissertation he tried to determine the nature of resistance of the Mignonette variety of lettuce. In so doing, he conducted field tests in which lettuce plants were exposed to naturally occuring *Thrips tabaci*, some of which were infective for TSWV. This indicates that there was still potential for TSWV outbreaks on lettuce at that time.

In other reports of TSWV on lettuce, Moller and Rogers (1960) tell of an outbreak in South Australia in which many growers lost more than half their crops in a period of two weeks. Docampo and Nome (1970) from Chile determined conclusively that the etiological agent causing death of lettuce plants in the zones of Quillota and San Felipe was in fact Tomato Spotted Wilt Virus. Boelma and Bolton (1984) listed TSWV among the important diseases of lettuce in South Africa.

Thrips - vector of TSWV

Only one family of insects, the Thripidae, is known to infect plants with TSWV (Pittman, 1927). Thrips are small insects approximately one millimeter in length. They are characterized by their rasping and sucking mouthparts (Fichter, 1966). Bald and Samuel (1931) reported on several factors in the relationship between thrips and TSWV. Thrips acquire TSWV during the larval stage by shallow feeding on infected plants and they are maximally infective 22-30 days afterward. Adult thrips can not become infective unless they have fed on virus-carrying plants while still in the larval stage.

For greatest thrips infectivity potential, an acquisition time of one to four days and an incubation period of four to twelve days is required. Once acquiring TSWV, thrips generally remain infective for life, but are unable to pass the virus on to their offspring (Sakimura, 1961).

Cho et al. (1986) list six separate reports in which six species of thrips have been identified as vectors of TSWV. They are: *Thrips tabaci* the onion thrips, *Frankliniella occidentalis* the western flower thrips, *Frankliniella fusca* the tobacco thrips, *Frankliniella schultzei* the common blossom thrips, *Scirtothrips dorsalis* the chillie thrips, and *Thrips setosus*. Only *Thrips tabaci*, *Frankliniella occidentalis*, and *Frankliniella schultzei* are present in Hawaii (Cho et al. 1986).

General features of TSWV

Tomato Spotted Wilt Virus is a RNA-containing virus with membrane-bound particles that are isometric in shape and 70-90 nm in size. The virus particles are found in leaf, stem, root, and petal cells (Ie, 1970). TSWV transmission in nature is only by thrips, but mechanical inoculation can be successful, especially when using reducing agents such as sodium sulfite in a pH 7 buffered inoculum (Best, 1968). Several of the characteristics of TSWV that differentiate it from other viruses are listed in Table 2.

Table 2. Characteristics of Tomato Spotted Wilt Virus (Best, 1968).

1). Transmission in nature only by the insect family *Thripidae*.

2). Thermal inactivation temperature of 46 degrees $\pm$ 1 degree C.

3). Short in vitro life of less than 12 hours in extracted plant sap, and less than 24 hours in buffer solution at pH 7 at room temperature.

4). Rapid inactivation at and below pH 5.5.

5). Rapid inactivation at redox potential of + 0.2 volts or greater at pH 7.

6). Extensive host range. Currently listed as 235 plant species (Cho et al. 1986).

Symptomatology of TSWV

TSWV was first reported as a disease of tomatoes (Brittlebank 1919). Therefore descriptions of the disease are often those symptoms that occur on tomatoes. These can be classified as necrotic and pigmented lesions and patterns, mild surface necrotic ringspots and etch patterns, yellows, and non-necrotic mottle or mosaic in greens (Best 1968). On lettuce TSWV symptoms are necrotic spots usually on one side of the leaf, twisted or distorted leaves, midrib pitting, stunting of growth, wilting and eventual collapse and death of plant (Table 3, Figure 1).

Figure 1. 'Manoa' lettuce infected with Tomato Spotted Wilt Virus. a. Early stage of infection. b. Late stage of infection.

Table 3. Symptomatology of Tomato Spotted Wilt Virus reported on lettuce.

1934	Tomkins and Gardner	Leaves have slight marginal wilting, necrotic spotting, and slight yellowing, usually on one side of the plant, with consequent tilting of the head toward the affected side. Lateral curvature of many leaves is produced.
1939	Harris	Older leaves on young plants curl or twist to one side. Very frequently within a week or ten days after infection the entire plant wilts and dies.
1940	Sakimura	Leaves have necrotic spots, necrotic blotching, distortion and yellowing.
1957	Borchers	Leaves show necrotic flecking, chlorotic and necrotic spotting, and midrib pitting.
1960	Moller and Rogers	Young plants turn yellow, hearts fail to develop, and eventually the plants wilt and flatten out.
1970	Docampo and Nome	Wilting of mature leaves, curling of young leaves, and final destruction of lettuce plant.

Table 3. Symptomatology of TSWV on lettuce (continued).

1980	Boelema and Bolton	Young plants become bent and twisted and may die within a few weeks. Older plants develop a large number of brown spots on the leaves, especially near the lower part of the midrib and along the main veins and the heads develop unevenly.
1986	Cho et al.	Necrotic brown leaf spots on one side of the plant becoming systemic and extending to the heart leaves, resulting in cessation of development and twisting of the plant to one side.

Control of TSWV

There are several different approaches towards control of TSWV. Best (1968) mentions four different methods:

1) Protective infection.
2) Reduction of vector influence.
3) Parasitism.
4) Use of resistant varieties.

Protective infection is the inoculation of a plant with a weak strain of virus to protect it from a more virulent strain. Best (1968) tried this technique with 500 tomato plants in two consecutive summers. Each year he mechanically inoculated half of the plants with a mild strain of TSWV virus he named E and the other half were left as controls. The plants were then left to natural exposure to TSWV infected thrips. The first summer showed no significant difference between control and infected plants in terms of total and marketable yield, but the second summer did show a significant difference with the protectively infected plants yielding higher in both categories. However, in all cases the protectively infected plants yielded lower than plants that were never infected by the virus.

Reducing the influence of the thrips vector can be accomplished by killing the thrips, by removing nearby plants favored by the thrips and which are hosts for TSWV, or by preventing the thrips from feeding on susceptible crop plants (Harris, 1939, Mau, 1986).

Perhaps the easiest way to kill thrips is by spraying an insecticide, but this generally isn't 100 percent effective. Some infective thrips will survive or others will come in from surrounding areas. Spraying of thrips might lower the amount of TSWV in the field, but it usually won't eliminate it, and thus may not be economically feasable (Best, 1968).

Weeds and wild plants allow the perpetuation of viruses and are often the source from which vectors spread the viruses (Bos, 1981). Mau (1986) therefore suggests removal of nearby weed host plants, roguing of infected crop plants, and removal of remaining host crop plants after final harvest to reduce disease incidence. Harris (1939) suggested similar procedures following the TSWV outbreaks in the Salinas Valley in the 1930's.

Thrips may be discouraged from feeding on susceptible crop plants by use of barriers. Mau (1986) reports on studies that show 90 percent of adult thrips trapped in lettuce fields were flying below 4 feet.

Parasitism is a control method Smith (1932) suggested since there are two cases on record in which hymenopterous parasites attacked thrips species.

Breeding for resistant varieties would seem to be the best long term solution for control of TSWV in crops where it can be economically justified and where resistant lines can be found (Bjorling, 1966).

Genetics of TSWV resistance in tomato

The initial resistance study in tomatoes was by Samuel et al. (1930). In their report they noted small fruit size and TSWV resistance were linked. Kikuta et al. (1946), using the single dominant gene for resistance found in Porter's strain of Lycopersicon pimpinnelifolium developed the resistant L. esculentum variety Pearl Harbor. Holmes (1948) reported resistance in the L. esculentum cultivars Manzana and Rey de los Tempranos. He determined this resistance was controlled by a single recessive gene.

Finlay (1953) reported on the inheritance of resistance to TSWV in tomatoes. He crossed the TSWV resistant tomato cultivars Pearl Harbor, Manzana, Rey de los Tempranos and Porter's strain of L. pimpinnelifolium to each other and to the susceptible L. esculentum cultivar Potentate. From the resulting F_1 and F_2 generations he determined the four tomato types were resistant to five out of ten classes of TSWV strains. Resistance was controlled by two dominant alleles at one locus and three independently inherited recessive genes. These were listed as SW_{1a}, SW_{1b}, sw_2, sw_3, and sw_4. Thus for maximum resistance to all five strains a tomato plant should have the genotype SW_{1a} SW_{1b}, sw_2 sw_2, sw_3 sw_3, sw_4 sw_4. However, Upreti (1983), who conducted a study similar to but not as extensive as Finlay's, postulated that resistance might be controlled by as many as four independent genes, two dominant and two recessive.

Unfortunately, the development of TSWV resistance in tomato cultivars has been hindered by the linkage between small fruit size and TSWV resistance (Best, 1968).

MATERIALS AND METHODS

Plant materials

The plant materials used for this research were the *Lactuca sativa* cultivars 'Green Mignonette','Tinto', and 'Batavia', and the Plant Inventory (PI) accessions 167128, 342510, 342517, 342522, and 342526. The characteristics of these materials are listed in Table 4.

'Manoa' is the name used in Hawaii for the cultivar 'Green Mignonette'. 'Manoa' is a heat tolerant, semi-heading type lettuce popular in Hawaii because of its dark green leaves, texture, and non-bitter taste. 'Manoa', since it is heat tolerant, is well adapted to the higher temperatures encountered at lower elevations in Hawaii, and can thus be grown year round in Hawaii. Unfortunately, 'Manoa' is highly susceptible to TSWV despite Borcher's (1957) report from California. Therefore, throughout the resistance trials in this research, 'Manoa' was used as a susceptible check.

The cultivar 'Tinto' and the P.I. accessions 342510, 342517, 342522, and 342526 are all semi-heading type lettuce, while P.I. 167128 is a Romaine type. These lines are included because they exhibited some resistance to TSWV in Hartmann's (personal communication) preliminary resistance screening trials.

'Batavia' is a leaf type lettuce acquired from Dennis Gonsalves in the Plant Pathology department of the

Table 4. Source, description and expected spotted wilt reaction of lettuce lines used as parents.

Lettuce line	Type	Seed color	Source	Reported TSWV reaction
Manoa	semi-heading	brown	U.H. Horticulture breeding strain	Susceptible
Tinto	semi-heading	white	E.J. Ryder, ARS Salinas, California	Resistant
Batavia	leaf	white	Cornell University Plant Pathology Dept.	Resistant
PI 167128	Romaine	white	Western Region Plant Introduction Station	Resistant
PI 342510	semi-heading	white	Western Region Plant Introduction Station	Resistant
PI 342517	semi-heading	brown	Western Region Plant Introduction Station	Resistant
PI 342522	semi-heading	brown	Western Region Plant Introduction Station	Resistant
PI 342526	semi-heading	brown	Western Region Plant Introduction Station	Resistant

D.W. Barton Lab of Cornell University, Geneva, New York. 'Batavia' is included because Gonsalves (personal correspondence) in 1984 indicated it was resistant to TSWV in his resistance screening trials.

Crossing procedure

The lettuce lines to be crossed were grown at the University of Hawaii-Manoa mauka campus facility, beginning in March, 1985. A soilless mix which contained 2 parts peat moss, 2 parts vermiculite, and 1 part perlite in 12" pots was used for growing the lettuce.

Lettuce plants reach flowering stage at approximately 3 months of age. When lettuce flowers open they are normally self-pollinated when the stigma picks up pollen as it grows through the anther sheath. If this pollen can be removed before the stigma forks and bends outward, self-pollination can be prevented. Therefore Ryder's (1974) method of applying intermittent mist during the time of anther dehiscence to wash away the pollen grains so they can not germinate on the receptive stigma was implemented. When this method of emasculation is used, about 95% hybrid seed can be expected.

The procedure used was to place plants which were approaching flowering and were to be used as females on a bench with intermittent mist. The mist nozzles were located above the lettuce flowers and were on for 30 seconds every 5 minutes.

When the flowers opened, usually for only a couple of hours in the morning, the mist would emasculate the flowers. The lettuce plants were removed from the mist when the stigmas had emerged through the anther sheaths and had begun to fork outwards. The flowers were dried off with a fan and pollinated with pollen from open flowers from the desired male line. Pollination was made by rubbing the pollen-covered stigmas of an open male flower over the stigmas of the female flower. Usually, about 8 flowers per female plant were pollinated each day. After being pollinated, each flower was tagged with the parental names and date of cross. To detect if any self-pollination was occurring, 1-3 flowers per plant were tagged but not pollinated. Thus if a non-pollinated flower on a plant produced seed, selfing would have occurred and the parentage of the seeds produced by the other flowers on that plant would be in doubt.

The lines reported as TSWV resistant, PI lines 342510, 342517, 342522, 342526, 167128, and cultivars 'Tinto' and 'Batavia' were all crossed to the susceptible 'Manoa' and to each other. Most crosses between the resistant parents and the susceptible 'Manoa' used 'Manoa' as the female parent and the other lines as male parents. In crosses between resistant parents, either parent was used as the female parent.

Some of the hybrid F_1 seeds were planted to produce F_2 seed while others were used for resistance testing.

Mechanical Inoculation procedures

Mechanical inoculation is the transfer of virus containing sap from one plant to another, generally by means of rubbing the inoculum over the leaves of the target plant.

The procedure used for inoculation tests in this study was as follows. Lettuce seeds were planted in the greenhouse in 12" pots with sterilized soil and a top layer of 2" of vermiculite containing a tablespoon of osmocote 10-30-10. Ten lettuce plants of each line were allowed to grow per test pot. Each week separate pots of 'Manoa' and Emilia fosbergii Nicolson, a common weed in Hawaii and a host of TSWV, were also planted for use as susceptible checks for testing the infectivity of the inoculum and as a source of inoculum.

When the plants reached 3 weeks of age they were inoculated with TSWV. Infected plant tissue for inoculum was obtained from previously inoculated Emilia leaves which showed mottling or from 'Manoa' lettuce which showed necrotic specking, each of which is a TSWV symptom for the respective plant. The buffer used for inoculation consisted of 100 ml of chilled 0.1 M potassium phosphate, pH 7, containing 0.1 g sodium meta-bisulfite (reducing agent) (Best, 1968). To make the inoculum, TSWV infected leaves were added to the buffer in a prechilled mortar. They were ground to a liquid consistency with a prechilled pestle, after which carborundum powder was added. The actual inoculation procedure was done by gently rubbing the leaves with a second prechilled pestle dipped in inoculum. Inoculations were done immediately after inoculum was prepared and were completed within 20-30 minutes. After inoculation, the pots were moved to a bench outside the greenhouse.

When plants became infected by this method, symptoms would start to appear 7-10 days after inoculation in _Emilia_ and two to three weeks after inoculation in lettuce.

The initial mechanical inoculations were made in 1978-80 at the UH Manoa mauka campus greenhouse facility using the above procedures. This test was conducted by R.W. Hartmann, R.T. Nagata, and K.T. Taniguchi. Testing at this site was discontinued in 1980 after the germplasm had been screened and a build-up of infected thrips had made it impossible to use the facility for other host plants such as tomato.

In 1984 the inoculation test was restarted in a new location, Pope greenhouse located on the UH Manoa campus. This location was selected because it was not used for other horticultural crops susceptible to TSWV. The intent of these inoculation attempts was to first establish the procedures whereby 'Manoa' could be routinely infected and then to retest some of the accessions of Hartmann et al. which had exhibited some resistance. Procedures used were the same as those used previously. However, 'Manoa' was not becoming regularly infected so numerous modifications to the previous procedure were tried. The carborundum was sprinkled on the leaves instead of being added to the inoculum, plants were left in the greenhouse after being inoculated, plants were placed in darkness for 24 hours before inoculation, plants were placed in a cool room (15^{o}C) after inoculation, plants were placed in the cool room and darkness 24 hours before inoculation, infected Manoa lettuce brought over from Maui was used as inoculum, the dilution of inoculum was varied, a soft bristle brush was used to apply the inoculum, the inoculum was washed off the leaves 10-15 minutes after inoculation, and experienced inoculators (Cho and Gonsalves, both professors of Plant Pathology) were used as inoculators.

In January, 1986 funds were received which permitted the testing of lettuce germplasm at Kula, Maui. The testing was done by mechanical inoculation in a greenhouse at the Kula branch Agriculture Experimental Station.

After some preliminary experiments in January and February of 1986 with different sources of inoculum (Nicotiana benthamiana and romaine lettuce), different dilutions of inoculum, and different inoculum applicators, inoculations at Kula followed the same procedure as first described in this section with 2 exceptions; a soft bristle brush (10-15 mm wide) was used instead of a pestle for inoculum application, and the carborundum was sprinkled over the leaves instead of being put in the inoculum.

At Kula necrotic local lesions appeared on inoculated leaves in one week, while systemic symptoms in infected plants began to show 2 or more weeks after inoculation.

Testing for resistance

There were six separate tests used for evaluating TSWV resistance in lettuce. In chronological order they are:

Test 1. UH Manoa, mauka campus greenhouse facility, initial screening, inoculation trial, 1979-1980.

Test 2. Hawaii Kai, Oahu, field trial of resistant parents and some F_1s, January - March, 1986.

Test 3. Kula, Maui Agriculture Station, resistant parent inoculation trial, March - June, 1986.

Test 4. Hawaii Kai, Oahu, field trial of resistant parents and some F_1s, March - June, 1986.

Test 5. Kula, Maui Agriculture Experimental Station, weekly inoculation trials of two resistant parents, July-September, 1986.

Test 6. Kula, Maui, Agriculture Experimental Station, inoculation trial of resistant parents, F_1s, and F_2s, August - October, 1986.

Test 1

Test 1 took place at the UH Manoa mauka campus greenhouse facility from March, 1979 to November, 1980. In this trial 112 lettuce varieties received from E.J. Ryder, 531 PI lines of _Lactuca_ _sativa_, and 55 PI lines of other species of _Lactuca_ were inoculated. Two pots with 10 plants per pot were planted for each accession. Three weeks later they were inoculated. 89 of the lines which appeared to be resistant in the first test were retested.

Test 2

Test 2 was held at the Enomoto farm in the Kamilonui valley in the southeastern part of Oahu near Hawaii Kai beginning in January, 1986. This site was chosen because of its high levels of TSWV on lettuce. Several adjoining farmers in the area had already stopped growing lettuce because of the persistent TSWV problem. This test took place on the only remaining farm still growing lettuce. The purpose of this test was to evaluate resistance in an actual field environment.

The lettuce plants for this test were grown from seed in 'Speedling' trays at the UH Manoa Mauka campus greenhouse. Seeds were planted December 25, 1985. On January 17, 1986 PI 167128, all of the crosses, and half of the other parental lines were mechanically inoculated with TSWV in the greenhouse to ensure there was virus in the planting and to see if there would be a difference in rate of infection between the inoculated and the uninoculated parent lines in the field. The seedlings were transplanted to the Hawaii Kai field January 23, 1986 (4 weeks after planting). This test included the resistant accessions from Hartmann and Gonsalves, the susceptible check 'Manoa', F_1 plants from crosses between the resistant parents and 'Manoa', and F_1 plants from some crosses between the resistant parents (Table 5).

The plants were planted in randomized order in 2 center rows 1 foot apart in a raised 4 foot bed. Border rows

Table 5. Parent lines and crosses transplanted January 23, 1986 to Kamilonui farm, Oahu and number of plants inoculated before transplanting (Test 2).

Line or cross	# of plants transplanted	# of plants inoculated
Manoa	10	5
Batavia	7	4
Tinto	9	5
167128	10	10
342510	12	6
342517	3	2
342522	8	4
342526	9	5
Manoa x Batavia	4	4
Manoa x Tinto	2	2
Manoa x 167128	1	1
Manoa x 342517	5	5
Manoa x 342522	3	3
Manoa x 342526	6	6
Batavia x 342522	9	9
Tinto x 342517	5	5
342517 x Tinto	5	5
Tinto x 342522	2	2
342522 x Tinto	5	5
342517 x 342526	10	10
342517 x 342522	5	5
342522 x 342517	4	4
342522 x 342526	9	9
Total	143	116

of approximately 6 week old 'Manoa' lettuce from a different field on the same farm were transplanted into the two outer rows of the same bed. Adjoining beds in the field were transplanted with green onions.

Plants were checked weekly for TSWV symptoms through March 19 (8 weeks) at which time all remaining plants had begun bolting. A rating scale of 1-5 was used for evaluation of TSWV infection. The # 1 was given to plants with no symptoms, # 2 to plants basically healthy, but with mild specking or spotting on the leaves, # 3 to plants with highly visible spots and necrotic regions (unmarketable plants) , # 4 to plants with large necrotic regions and stunting of growth, and # 5 to plants very severely affected or dead. Plants that did not recover from transplant shock, crosses that appeared to be selfs, and plants that were damaged by some means other than TSWV were not rated.

Test 3

Test 3 was an inoculation attempt held at Kula, Maui. The first part of this test included the same parental lines used in Test 2 ('Tinto', PI 167128, PI 342510, PI 342517, PI 342522, PI 342526, 'Batavia', and 'Manoa'). Two pots with ten seeds per pot were planted for each line except 'Manoa' which had ten seeds planted in a single pot. Seeds were planted February 25, 1986. Plants were inoculated March 18, 1986 by C. Yoshii and again March 31. R.W. Hartmann and C. Yoshii evaluated the plants on March 24, March 31, and

April 4, 1986, recording the visual symptoms and number of systemic plants.

The second part of Test 3 included all the parents except 'Manoa' and 'Batavia'. Seed from 2-4 individual plants per parent line were planted in 72 compartment 'Speedling' trays. All seeds of each parent line were planted at the same time. The different lines were planted March 24, April 2, 8, 10, 1986. For inoculations each tray was divided into 3 sections. On each section a different dilution of inoculum was used. The dilutions were none, 1:1, and 1:3. Plants were inoculated at 2-3 weeks of age, reinoculated 1 week later, and evaluated for systemic symptoms 3 weeks after the first inoculation.

<u>Test 4</u>

Test 4 was held at the same Hawaii Kai location as Test 2. Seeds were planted in the UH mauka greenhouse on April 1, 1986. Plants were not inoculated prior to being transplanted to the farm on April 29, 1986.

All parental lines used in Test 2 with the exception of PI 342510 were planted. The F_1s used in Test 2 which still had more seed were replanted, and some additional F_1s produced after the start of Test 2, were also planted (Table 6).

Table 6. Parent lines and crosses transplanted April 29, 1986 to Kamilonui, farm, Oahu (Test 4).

Line or cross	# of plants transplanted
Manoa	10
Batavia	6
Tinto	16
167128	2
342517	11
342522	10
342526	10
Manoa x 342526[z]	4
Tinto x 342517[z]	8
167128 x 342522	2
342517 x 342522[z]	6
342517 x 342526[z]	4
342522 x 342526[z]	4
342517 x Batavia	4
342526 x Tinto	4
Batavia x Tinto	4
Batavia x 342522[z]	3
Total	108

z Crosses that were also used in Test 2.

No border rows of 'Manoa' were put around the planting, but the surrounding beds were again planted with green onions. Plants were checked weekly through June 18 (7 weeks) at which time all remaining plants had begun bolting. The (1-5) scale was again used for rating.

Test 5

This test was a series of inoculation trials at the Kula station of the two most resistant parental lines of the three previous tests, 'Tinto' and PI 342517, and the susceptible 'Manoa'. It took place from July to September, 1986. Seeds were planted in 'Speedling' trays with 72 compartments. One third of the compartments were used for each parent. Plants were inoculated by C. Yoshii at about 3 weeks of age (Table 7). Unlike in Test 3, plants were not reinoculated. TSWV infection was evaluated by C Yoshii 3 weeks after inoculation. The number of plants that showed a systemic infection were recorded.

Test 6

Test 6 included the parental lines, 'Manoa', 'Tinto', and PI 342517, the F_1 crosses between them, and the F_2s from these crosses. Seeds were planted in every other compartment of the 72 compartment 'Speedling' trays (36 plants per tray) on August 19, 1986 at Kula, Maui. One tray of each of the parents and F_1s was planted, and three trays of each F_2. Each F_2 tray included seed produced

Table 7. Planting and inoculation dates for trials of Tinto, PI 342517, and Manoa at Kula, Maui July - September, 1986 (Test 5).

Trial	Planting Date	Inoculation Date
1	7/09/86	7/29/86
2	7/16/86	8/06/86
3	7/24/86	8/13/86
4	8/14/86	9/10/86
5	8/20/86	9/10/86

from an individual F_1 plant, except for the seeds of 'Manoa' x 'Tinto' which all came from the same plant (Table 8). The seedlings were inoculated with TSWV on September 8, half by C. Yoshii and half by myself, and were evaluated September 30. The plants were rated for TSWV 3 weeks after inoculation by two different methods. I recorded whether plants showed any spotted wilt symptoms. C. Yoshii recorded only plants that showed systemic infection. Each of us evaluated all the plants.

Table 8. Tinto, PI 342517, Manoa, and their F_1 and F_2s inoculated September 8, 1986 at Kula, Maui (Test 6).

Population	Rep	# of plants
Parental lines		
Manoa		36
Tinto		36
PI 342517		36
F1's		
Manoa x Tinto		34
PI 342517 x Tinto		21
Manoa x PI 342517		7
F2's		
Tinto x PI 342517	(1)	36
Tinto x PI 342517	(2)	36
Tinto x PI 342517	(3)	36
PI 342517 x Manoa	(1)	36
PI 342517 x Manoa	(2)	36
PI 342517 x Manoa	(3)	36
Manoa x Tinto	(1)	35
Manoa x Tinto	(2)	36
Manoa x Tinto	(3)	36

RESULTS and DISCUSSION

Crosses which produced seeds

The crosses between the susceptible 'Manoa' and the resistant parents or between two resistant parents were tried in all combinations, 34 of which were successful (Table 9). Plants from the seeds of these crosses were checked to insure they were F_1s when grown for testing or for making F_2s. Leaf shape, leaf color, and anthocyanin pigmentation of the leaves and stems were the characteristics used for determination of whether the plants were selfs or hybrids.

In tests 2, 4, and 6, F_1 seed used came only from female plants that did not produce seed from tagged unpollinated flowers. In tests 2 and 4, there were 3 selfs out of 118 plants. In test 6 there were no selfs among 62 seeds. Plants grown out to produce F_2 seed had 2 out of 25 plants that were determined to be selfs.

These results show a high percentage of true crosses were made by the method outlined by Ryder and Johnson (1974). However, it should be noted that several times seeds were produced on unpollinated flowers, while other times no seed was produced even on pollinated flowers.

Inoculation

Hartmann, Nagata, and Taniguchi mechanically inoculated lettuce lines at the mauka greenhouse facility in 1979-80.

Table 9. Crosses which produced seed, number of seed grown, and number of accidental selfs obtained.

Female parent	Male parent	# of crosses produced seed	# seed grown	# selfs[z]
Manoa ----------	Tinto	8	39	0
Manoa ----------	PI 167128	8	3	1
Manoa ----------	PI 342510	2	3	1
Manoa ----------	PI 342517	4	17	1
Manoa ----------	PI 342522	2	3	1
Manoa ----------	PI 342526	4	8	0
Manoa ----------	Batavia	2	4	0
Tinto ----------	Manoa	4	5	0
Tinto ----------	PI 342517	2	14	0
Tinto ----------	PI 342522	1	3	0
Tinto ----------	PI 342526	2	3	0
PI 167128 ------	PI 342510	3	0	
PI 167128 ------	PI 342522	3	1	1
PI 167128 ------	PI 342526	3	0	
PI 167128 ------	Tinto	1	0	
PI 342510 ------	PI 167128	2	0	
PI 342510 ------	PI 342517	1	0	
PI 342510 ------	PI 342522	6	0	
PI 342510 ------	PI 342526	1	0	
PI 342510 ------	Tinto	2	0	
PI 342517 ------	Manoa	2	2	0
PI 342517 ------	PI 342522	5	11	0
PI 342517 ------	PI 342526	8	13	0
PI 342517 ------	Tinto	9	29	0
PI 342517 ------	Batavia	2	5	0
PI 342522 ------	PI 167128	1	0	
PI 342522 ------	PI 342510	1	0	
PI 342522 ------	PI 342517	1	8	0
PI 342522 ------	PI 342526	9	14	0
PI 342522 ------	Tinto	8	5	0
PI 342522 ------	Batavia	1	0	
PI 342526 ------	Tinto	4	4	0
Batavia --------	PI 342522	6	12	0
Batavia --------	Tinto	2	6	0
		---	---	--
Total		120	205	5

z Plants confirmed F_1 while grown for F_2 seed, or when used in tests 2, 4, 6.

This site and technique was also used concurrently by Upreti (1983) to test TSWV on tomatoes. Upreti reported encouraging infective thrips to aid in his inoculations. Hartmann (personal communication) also noted that thrips were prevalent in that greenhouse, and caused high levels of TSWV infection on uninoculated lettuce, tomatoes, and green peppers grown in it.

When inoculations were attempted using the same procedures in Pope greenhouse in 1984, results were different. At first approximately 70 % of the Emilia plants and none of the 'Manoa' lettuce plants became infected with TSWV. After this initial period, 80 - 100 % of the Emilia plants consistently became infected, but never more than 40 % and often none of the 'Manoa' lettuce plants. The one exception to this scenario was a three week period in December when 70 %, 60 %, and 70 % of the 'Manoa' lettuce plants were infected.

The Emilia plants were always inoculated after the lettuce plants, but had nearly 100 % infection. This indicates the inoculum used was indeed infective, but the TSWV did not produce symptoms in the 'Manoa' lettuce plants. None of the modifications of the inoculation technique increased the amount of infection.

The inoculations at Pope greenhouse never did produce satisfactory results and were finally discontinued.

Inoculations were started at Kula because it was reported they worked very easily there (Cho, personal communication). At Kula the inoculations were very effective. The susceptible 'Manoa' could be routinely infected with essentially the same inoculation technique as used on Oahu.

Since all three sites used essentially the same inoculation techniques, the difference in success rates must have been due to other factors, most likely the presence or absence of thrips and the differences in temperature. The greenhouses at the Manoa mauka campus and at Pope lab would be expected to have about the same temperatures, so the major difference between them was the presence of thrips at the mauka site. Thrips were never seen in Pope. Thus, the thrips probably did most of the inoculation at the mauka facility. Without the thrips, mechanical inoculations at the mauka facility would probably have been as ineffective as those at Pope greenhouse.

At the higher elevation of the Kula greenhouse temperatures were considerably lower than the Oahu greenhouses. Since there were no thrips there either (thrips were never found in traps placed in the greenhouse), the temperature was the reason for successful infection rates at Kula compared to Pope greenhouse. It is hypothesized that at the Kula location with temperatures that rarely exceeded 40°C, the virus which has a low inactivation temperature of 46°C $\pm$ 1°C (Best, 1968) would be less likely to be inactivated.

Evaluation of Resistance in Parental Lines

Test 1

The screening program in 1979-80 identified 89 lines of lettuce that exhibited a lower level of TSWV infection than 'Manoa'. When they were retested, six lines of Lactuca sativa appeared to have some resistance (Tables 10 and 11). Seed was saved and crosses were made with these lines. However, the reliability of this test was questionable because 'Manoa' showed such a range of infection throughout it (Hartmann, personal communication).

Test 2

Following Test 1, crosses were made and carried out to the F_3 generation. These plants were grown out at the Poamoho agriculture experimental station on Oahu which was free of TSWV on lettuce. Unfortunately, they were nearly all destroyed by Hurricane Iwa in November, 1982. There was never any confirmation of resistance made in either the lines or crosses. Testing was reattempted in Pope greenhouse in 1984 to confirm or deny resistance, but it was unsuccessful.

Therefore arrangements were made to begin testing at Kula, Maui. This location is not ideal because of the logistics, so it was decided to see if a field test on Oahu could be used successfuly. This was Test 2 held in the Kamilonui Valley. The parent and F_1 plants were first examined 6

Table 10. Number of Tinto, PI 167128, PI 342510, PI 342517, PI 342522, PI 342526, and Manoa plants infected with Tomato Spotted Wilt Virus in 1979-1980 screening trial at the mauka Manoa greenhouse (Test 1).

Lettuce line	Date approximately 3 weeks after inoculation and # of plants infected			
	8/23/79	11/01/79	12/13/79	7/02/80
Tinto	7/20	-	4/10	-
PI 167128	-	1/10	-	-
PI 342510	-	-	-	2/10
PI 342517	-	-	-	2/10
PI 342522	-	-	-	0/10
PI 342526	-	-	-	-
Manoa	15/20	1/10	4/10	1/10
Most severely infected line	16/20	9/10	8/10	10/10

Lettuce line	7/08/80	8/21/80	9/04/80
Tinto	-	2/10	-
PI 167128	-	5/9	-
PI 342510	-	-	3/10
PI 342517	-	-	3/10
PI 342522	-	-	0/10
PI 342526	2/10	-	2/10
Manoa	8/10	9/10	7/10
Most severely infected line	10/10	10/10	10/10

Table 11. Summary of 1979-1980 lettuce screening trials (Test 1).

Lettuce line	% range of infected plants over several inoculation dates	# of trials	TSWV classification
Tinto	20 - 40 %	3	resistant
PI 167128	10 - 56 %	2	resistant
PI 342510	20 - 30 %	2	resistant
PI 342517	20 - 30 %	2	resistant
PI 342522	0 %	2	resistant
PI 342526	20 %	2	resistant
Manoa	10 - 90 %	7	susceptible
Most severely infected line	80 -100 %	7	susceptible

days after transplanting on January 29, 1986. At this time thrips damage was evident throughout the entire planting. The first symptoms of TSWV appeared on February 11. Every week thereafter more plants developed TSWV symptoms until the eighth week when all remaining plants had bolted. Differences in rate of infection between lines were greatest at 7 weeks after transplanting.

The ratings for the seventh week were analyzed to see if the mechanical inoculations had any effect. The infection ratings between the inoculated and uninoculated parents showed no significant difference in a t test at the 5% level (Table 12). This would indicate the pre-transplant inoculations had no effect, and that infection was a result of the thrips present in the field. This agrees with the lack of inoculation success at Pope greenhouse.

The results of this experiment (Table 13) show a range of infection, but no clear division between resistant and susceptible lines. Of the parents, 'Tinto' exhibited the highest level of resistance, and 'Batavia' the lowest. However, the only significant difference among parents was between these two lines. 'Manoa' was very close to 'Batavia' but not significantly different from 'Tinto' or any of the other parents. No conclusions could be made from this test, except perhaps that 'Batavia' is not resistant.

TSWV on lettuce in Hawaii has a normal cycle of high disease pressure during the warm summer and early fall months, declining pressure during the cooler late fall and

Table 12. Tomato Spotted Wilt Virus ratings of inoculated and uninoculated parent lines and crosses at Kamilonui, Oahu on March 12, 1986 for Test 2 (7 weeks after transplanting).

Line	# of plants inoculated	Mean rating	# of plants uninoculated	Mean rating
Batavia	3	4.33 z	3	4.67
Manoa	5	3.40	5	4.20
Tinto	5	2.40	3	1.67
PI 342510	5	4.40	6	3.33
PI 342522	3	2.33	4	3.00
PI 342526	4	4.00	4	3.00
Totals	25	3.48 ns y	25	3.36

Z Disease rating 1-5, class 1 is most resistant.
Y Non-significant inoculation effect by t test at 5% level.

Table 13. Tomato Spotted Wilt Virus ratings of parent lines and crosses at Kamilonui, Oahu on March 12, 1986 for Test 2 (7 weeks after transplanting).

Lettuce line or cross	# of plants rated	Mean[y]
PI 342517 X Tinto[z]	10	2.00 A[x]
Tinto	8	2.13 A
PI 342522	7	2.71 AB
PI 342517 X PI 342526	9	2.78 AB
PI 342522 X Tinto[z]	6	3.00 AB
PI 342517 X PI 342522[z]	9	3.00 AB
PI 342522 X PI 342526	8	3.13 AB
Manoa X PI 342517	5	3.40 AB
PI 342526	8	3.50 AB
Manoa X PI 342526	3	3.67 AB
PI 342517	3	3.67 AB
PI 342510	11	3.82 AB
PI 167128	10	4.00 AB
Manoa	10	4.00 AB
Batavia X PI 342522	8	4.00 AB
Batavia	6	4.50 B

Z Includes reciprocal crosses.
Y Disease rating 1-5, class 1 is most resistant, rated 7 weeks after transplanting at marketable maturity.
X Mean separation by Waller-Duncan multiple range test, 5% level.

winter months, and an increase in the spring when the weather begins to warm again. According to this cycle, TSWV pressure would be low in January when this test was set out. However, in this unique location, TSWV has been persistent even during the cooler months, as evidenced by TSWV on lettuce plants in several fields on the farm.

Test 3

This is the first test using mechanical inoculation at Kula. In this test all of the parental lines were severely infected with very little difference between them (Table 14). At three weeks after inoculation only 'Tinto', PI 342517, and 'Batavia' had any plants that did not show symptoms. R.W. Hartmann ranked the lines on severity of symptoms. He ranked 'Tinto' as best and PI 342517 as second best, the rest were indistinguishable from each other.

Obviously this test was too severe to detect resistance that might be present in these lines. So, it was thought that perhaps diluted inoculum might work. Seed saved from different plants were also tested to see if this made a difference.

Table 15 shows the results of the second part of this test. No difference was detected between seed obtained from separate plants of the same line, except possibly PI 342522, or in the 3 dilutions of inoculum. Again, nearly all plants of the supposedly resistant lines became affected.

Table 14. Tomato Spotted Wilt Virus infection on parental lines in March, 1986 at Kula, Maui (Test 3).

Lettuce line	# plants infected 3 weeks after inoculation	Comments
Tinto	17/20	Some plants unaffected, less severe symptoms.
PI 167128	20/20	All severely systemic.
PI 342510	20/20	All severely systemic.
PI 342517	19/20	Most systemic, but less severe symptoms.
PI 342522	3/3	All severely systemic.
PI 342526	19/19	All severely systemic.
Manoa	10/10	All severely systemic.
Batavia	19/20	All except 1 severely systemic.

Table 15. Tomato Spotted Wilt Virus infection of single-plant progeny of PI 342510, PI 342517, PI 342522, PI 342526, and Tinto at different inoculation dilutions at Kula, Maui (Test 3).

Line	Planting date	Inoc. date	Dilution 0	1:1	1:3	Total % infected
PI 342510 #1	4/10	5/2	94(18)	83(18)[z]	89(18)	89(54)
PI 342510 #2	"	"	94(18)	89(18)	100(18)	94(54)
Total % infected			94(36)	86(36)	94(36)	
PI 342517 #1	4/10	5/2	78(18)	67(18)	67(18)	70(54)
PI 342517 #2	"	"	89(18)	78(18)	72(18)	80(54)
PI 342517 #3	"	"	89(18)	78(18)	72(18)	80(54)
Total % infected			85(54)	74(54)	70(54)	
PI 342522 #1	4/8	4/24	43(14)	42(14)	64(14)	50(42)
PI 342522 #2	"	"	83(18)	61(18)	61(18)	69(54)
Total % infected			67(32)	53(32)	63(32)	
PI 342526 #1	4/2	4/17	78(18)	83(18)	89(18)	83(54)
PI 342526 #2	"	"	75(18)	83(18)	89(18)	80(54)
PI 342526 #3	"	"	78(18)	89(18)	72(18)	80(54)
PI 342526 #4	"	"	94(18)	83(18)	78(18)	85(54)
Total % infected			79(72)	85(72)	82(72)	
Tinto #1	3/24	4/10	78(18)	72(18)	89(18)	80(54)
Tinto #2	"	"	50 (8)	75 (8)	63 (8)	63(24)
Tinto #3	"	"	88 (8)	63 (8)	75 (8)	75(24)
Total % infected			82(34)	71(34)	79(34)	

Z Percentage of plants infected 3 weeks after inoculation, and (number) of plants tested.

Test 4

Since the first field test at Hawaii Kai resulted in a range of infection but few significant differences, it was repeated. Differences in rate of infection between lines were again greatest at 7 weeks, and thus the ratings for the seventh week were analyzed. In this second test (Table 16) PI 342517 and 'Tinto' were the most resistant parents and both differed significantly from 'Manoa'. 'Batavia' and PI 167128 were the most susceptible. Both of these along with PI 342522, PI 342526, and 'Manoa' formed a group which did not show any significant differences from each other. Also worth noting, the crosses PI 342517 x 'Tinto', PI 342517 x 'Batavia', and PI 342517 x PI 342522 were significantly more resistant than 'Manoa'. Only one cross (PI 342522 x PI 342526) that did not include 'Tinto' or PI 342517 showed a significant difference from 'Manoa'.

Thus, it seems 'Tinto' and PI 342517 are the most resistant. 'Tinto' was the most resistant in tests 2 and 4. PI 342517 was second most resistant in Test 4, but not in Test 2 in which only 3 plants were tested. Additionally, the cross of these two had the lowest infection rating of any line or cross in both tests 2 and 4. In Test 3, although not very resistant, they were observed to be the best and second best.

PI 167128 and 'Batavia' are not resistant. They never showed any more resistance than 'Manoa' in any of the tests. PI 342522 and PI 342526 may be somewhat intermediate

Table 16. Tomato Spotted Wilt Virus ratings of parent lines and crosses at Kamilonui, Oahu on June 18, 1986 (7 weeks after transplanting) (Test 4).

Lettuce line or cross	# of plants rated	Mean[Y]
PI 342517 X Tinto[Z]	8	1.63 A[X]
Tinto	16	1.75 A
PI 342517 X Batavia	4	1.75 A
PI 342517	10	1.80 A
PI 342517 X PI 342522[Z]	6	1.83 AB
PI 342522 X PI 342526	4	2.00 AB
Batavia X Tinto	4	2.25 ABC
PI 342526 X Tinto	4	2.50 ABCD
PI 342517 X PI 342526	4	3.00 BCD
Batavia X PI 342522	3	3.00 BCD
Manoa	9	3.33 CDE
Manoa X PI 342526	3	3.33 CDE
PI 342522	10	3.40 CDE
PI 342526	10	3.50 DE
Batavia	6	3.50 DE
PI 167128	4	4.50 E

Z Includes reciprocal crosses.
Y Disease rating 1-5, class 1 is most resistant, rated 7 weeks after transplanting at marketable maturity.
X Mean separation by Waller-Duncan multiple range test, 5% level.

for resistance. PI 342522 ranked higher than 'Manoa' for resistance in Test 2, but not in Test 4. PI 342526 was slightly higher than 'Manoa' in Test 2, but lower in Test 4. The cross between them was significantly more resistant than 'Manoa' in Test 4 but not in Test 2. PI 342510 ranked close to 'Manoa' in Test 2, but was not used in Test 4. It does not seem to have any resistance, either.

Thus, it was decided to concentrate only on 'Tinto' and PI 342517 as possible sources of resistance. Therefore, the remaining tests included only 'Tinto', PI 342517, and 'Manoa'.

Test 5

The overall results of the inoculation trials of Test 5 (Table 17) clearly show 'Tinto' and PI 342517 are significantly more resistant than the susceptible 'Manoa'. However, the percentage of infected plants in each line did vary from week to week, and in the third and fifth trials both resistant parents showed no significant difference from 'Manoa' (Table 18 and Figure 2). Seed for the fourth trial was planted one week earlier, but inoculated on the same day as the fifth trial. When these 2 trials are compared, the fourth trial, which was inoculated 4 weeks after planting showed a significant difference between all three lines, while the fifth trial, which was inoculated 3 weeeks after planting, showed no significant difference between lines.

Table 17. Tomato Spotted Wilt Virus infection rates of Tinto PI 342517, and Manoa from July - September, 1986 at Kula, Maui (Rated 3 weeks after inoculation) (Test 5).

Lettuce line	# of plants	% infection
Tinto	106	19.8 A[z]
PI 342517	120	25.0 A
Manoa	120	70.0 B

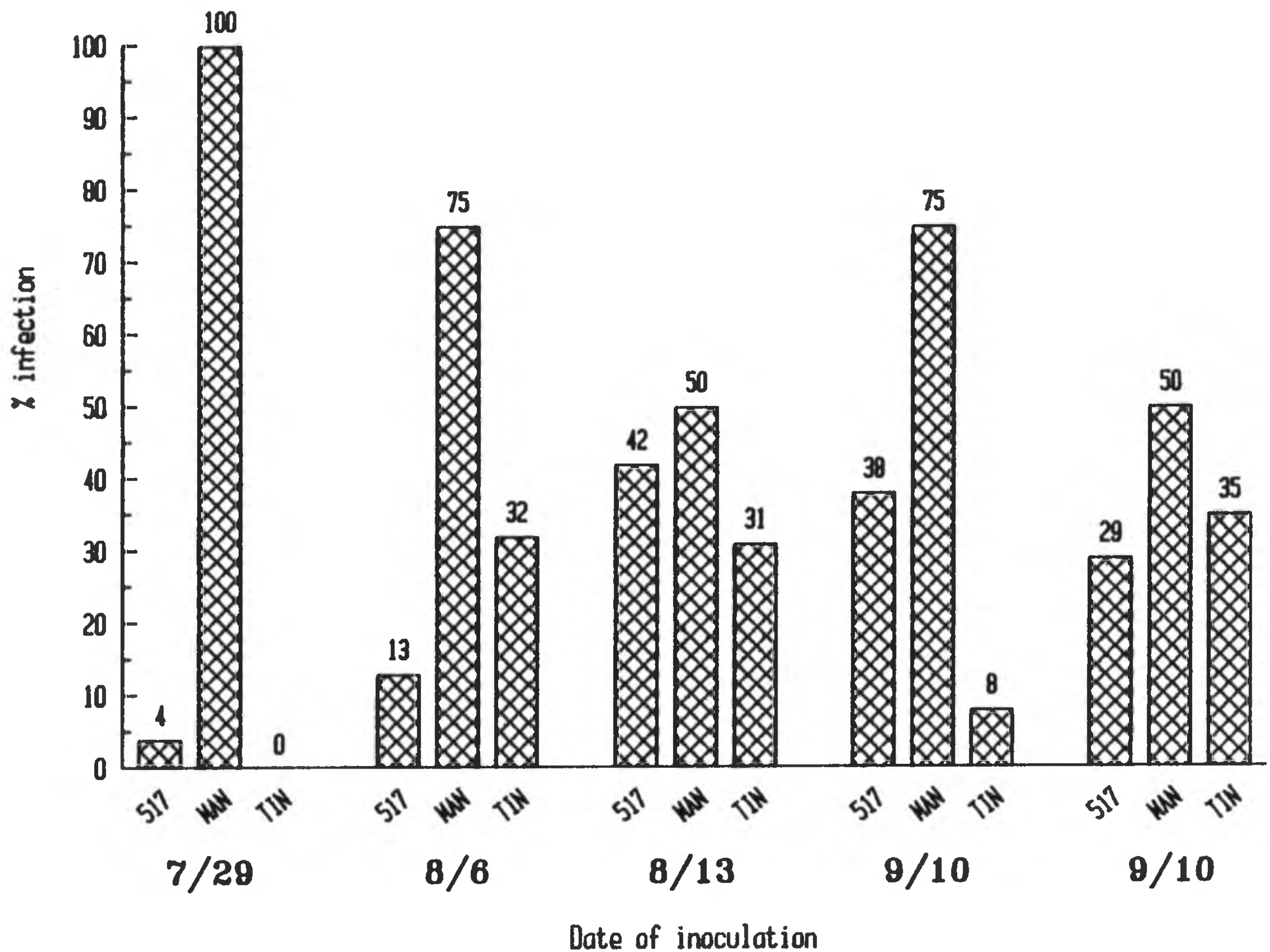
Z Mean separation by Waller-Duncan multiple range test, 5% level.

Table 18. Results of 5 individual trials summarized in table 17 (Test 5).

Lettuce line	# of plants	% infection
Trial 1. Inoculation date 7/29/86		
Tinto	24	0.0 A[Z]
PI 342517	24	4.2 A
Manoa	24	100.0 B
Trial 2. Inoculation date 8/06/86		
Tinto	22	31.8 A
PI 342517	24	12.5 A
Manoa	24	75.0 B
Trial 3. Inoculation date 8/3/86		
Tinto	16	31.3 A
PI 342517	24	41.7 A
Manoa	24	50.0 A
Trial 4. Inoculation date 9/10/86	Planting date 8/13/86	
Tinto	24	8.3 A[Z]
PI 342517	24	37.5 B
Manoa	24	75.0 C
Trial 5. Inoculation date 9/10/86	Planting date 8/20/86	
Tinto	20	35.0 A
PI 342517	24	29.2 A
Manoa	24	50.0 A

Z Mean separation by Waller-Duncan multiple range test, 5% level.

Figure 2. Maui Parental trials July - August, 1986 (Test 5).



This could indicate age of plant when inoculated has some influence on infection rate.

The percentage of infected plants in Test 5 was generally lower than in Test 3. Possibly, this is because the Test 3 plants were reinoculated one week after the first inoculation, while Test 5 plants were not. Reinoculation may neutralize much of the resistance found in 'Tinto' and PI 342517. The week to week variability can not be easily explained. It could be due to climatic effects on the condition of the host plants or titre of the inoculum obtained from the source plants, or perhaps other factors, as well.

Test 6

In this test infection was evaluated by two different methods. By the first, the systemic symptom method, plants were rated as infected when they showed obvious systemic symptoms (Table 19). In the second, the obvious symptom method, plants were rated on the basis of whether or not they had any TSWV symptoms other than local lesions on inoculated leaves (Table 20). The first method was used by C. Yoshii and is the same as used in tests 3 and 5. I used the second method to try for more precision in the determination of the onset of infection. Both evaluation methods resulted in a significant difference between the resistant parents, 'Tinto' and PI 342517, and the susceptible 'Manoa'. However, the obvious symptom method showed higher levels of infection for all entries, as well as a greater range of infection (11 - 72% vs. 0 - 53%). On the other hand, for the 3 repetitions of the 'Manoa' x 'Tinto' F_2 (which all came from the same F_1 plant), although there was no significant difference by either method, there was a range of 14.3% in the systemic symptom method, but only 7.5% in the obvious symptom method. Thus, although the obvious symptom method showed a larger range of infection overall, it showed a smaller infection range for the 'Manoa' x 'Tinto' F_2 plants. This would indicate the obvious symptom method not only detected infection more accurately, but also had a higher level of consistency. Therefore, when discrepancies appeared between the two methods in the resistance evaluation of the F_1 and F_2 populations, the conclusions based on the obvious symptom method were considered to have higher validity.

Table 19. Rate of Tomato Spotted Wilt Virus infection of Tinto, PI 342517, Manoa, and their F_1s and F_2s at Kula, Maui (Rated by systemic symptom method 3 weeks after inoculation) (Test 6).

Lettuce line or cross	# of plants	% infection
PI 342517	36	00.0 A[z]
PI 342517 X Manoa (F_1)	7	00.0 A
Tinto X PI 342517 (F_2) B	36	05.6 AB
Tinto	36	08.3 AB
Tinto X PI 342517 (F_2) C	36	11.1 ABC
Manoa X Tinto (F_2) C	35	14.3 ABC
Tinto X PI 342517 (F_1)	20	15.0 ABC
Tinto X PI 342517 (F_2) A	36	19.4 ABCD
Manoa X Tinto (F_2) B	36	22.2 BCD
Manoa X Tinto (F_1)	34	29.4 CD
Manoa X Tinto (F_2) A	36	30.6 CD
PI 342517 X Manoa (F_2) A	36	36.1 DE
PI 342517 X Manoa (F_2) B	36	36.1 DE
Manoa	36	52.8 E

Z Mean separation by Waller-Duncan multiple range test, 5% level.

Table 20. Rate of Tomato Spotted Wilt Virus infection of Tinto, PI 342517, Manoa, and their F_1s and F_2s at Kula, Maui (Rated by obvious symptom method 3 weeks after inoculation) (Test 6).

Lettuce line or cross	# of plants	% infection
PI 342517	36	11.1 A[z]
Tinto X PI 342517 (F_1)	20	20.0 AB
Tinto X PI 342517 (F_2) C	36	25.0 ABC
Tinto X PI 342517 (F_2) B	36	25.0 ABC
PI 342517 X Manoa (F_1)	7	28.6 ABC
Tinto	36	30.6 ABC
Manoa X Tinto (F_2) A	36	31.4 ABC
Tinto X PI 342517 (F_2) A	36	33.3 ABC
Manoa X Tinto (F_2) B	36	36.1 ABC
Manoa X Tinto (F_2) C	35	38.9 BC
PI 342517 X Manoa (F_2) B	36	38.9 BC
Manoa X Tinto (F_1)	34	44.1 BC
PI 342517 X Manoa (F_2) A	36	47.2 CD
Manoa	36	72.2 D

Z Mean separation by Waller-Duncan multiple range test, 5% level.

Evaluation of Resistance in Crosses

In Test 6 disease ratings for 'Tinto', PI 342517, their F_1s and F_2s were not significantly different from one another, and all were significantly different from 'Manoa' (Table 19, 20 and Figure 3). This suggests 'Tinto' and PI 342517 have the same gene or genes for resistance.

In the cross of 'Manoa' x 'Tinto' (Figure 4) the F_1 was significantly different from 'Manoa' but not from 'Tinto'. The F_2s were not significantly different from each other, from the F_1 or from 'Tinto', but they were significantly different from 'Manoa'. This evaluation suggests 'Tinto' has a dominant gene or genes for resistance.

In the cross of 'Manoa' x PI 342517 (Figure 5) the F_1 was significantly different from 'Manoa' but not from PI 342517. The F_2s were not significantly different from each other or from the F_1. The two F_2 populations were both significantly different from PI 342517, but only one was also significantly different from 'Manoa'. The third population of F_2s was not rated because the plants were not an F_2 population, but selfs of 'Manoa'. Since the F_1s from the cross of 'Manoa' x PI 342517 were resistant, and the F_2s segregated for susceptibility, implications are that PI 342517 also has a dominant gene or genes for resistance.

Figure 3. Obvious symptom method comparison of PI 342517, Tinto, and their progeny to Manoa (Test 6).

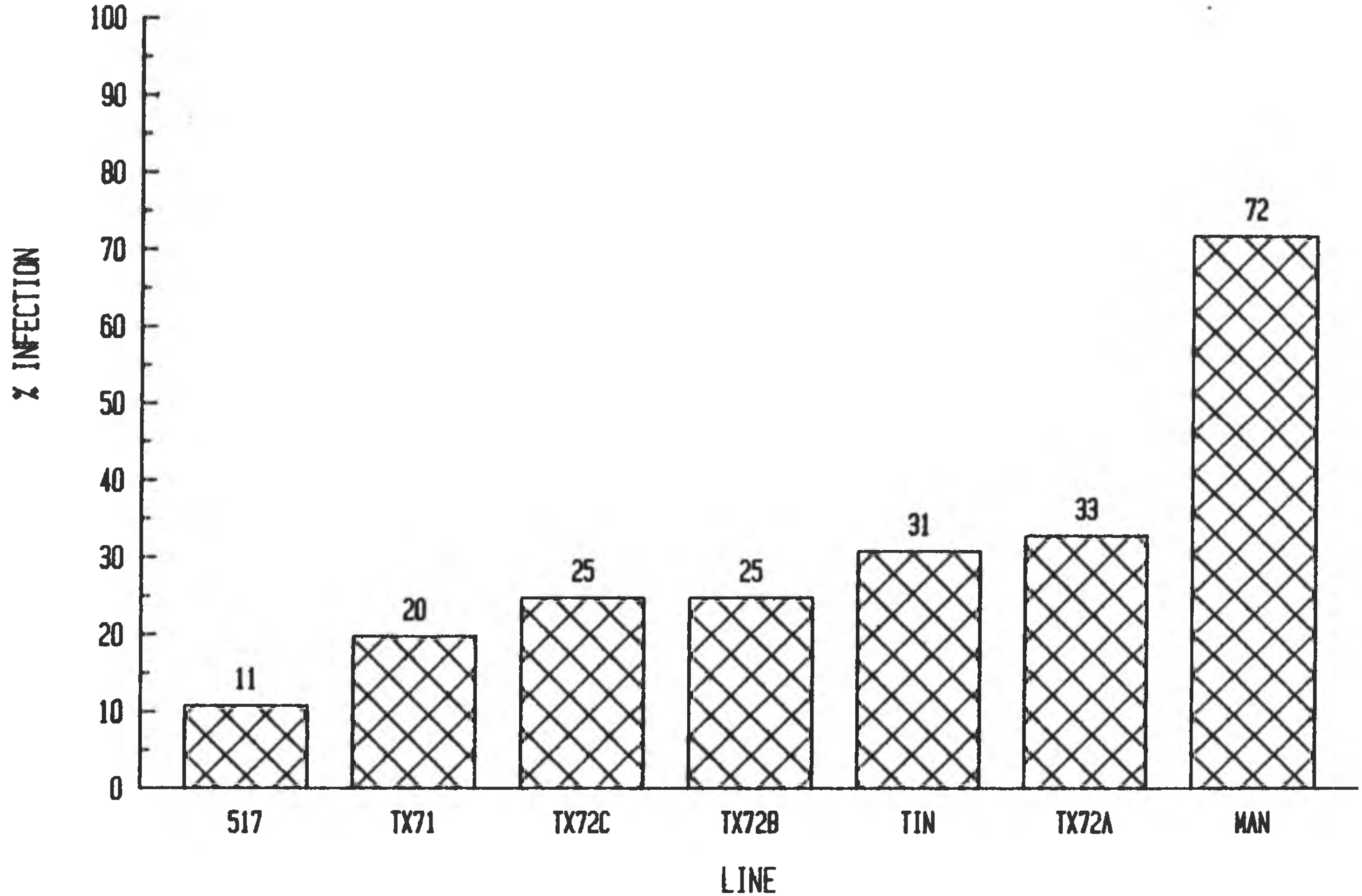


Figure 4. Obvious symptom method comparison of Tinto, Manoa, and their progeny (Test 6)

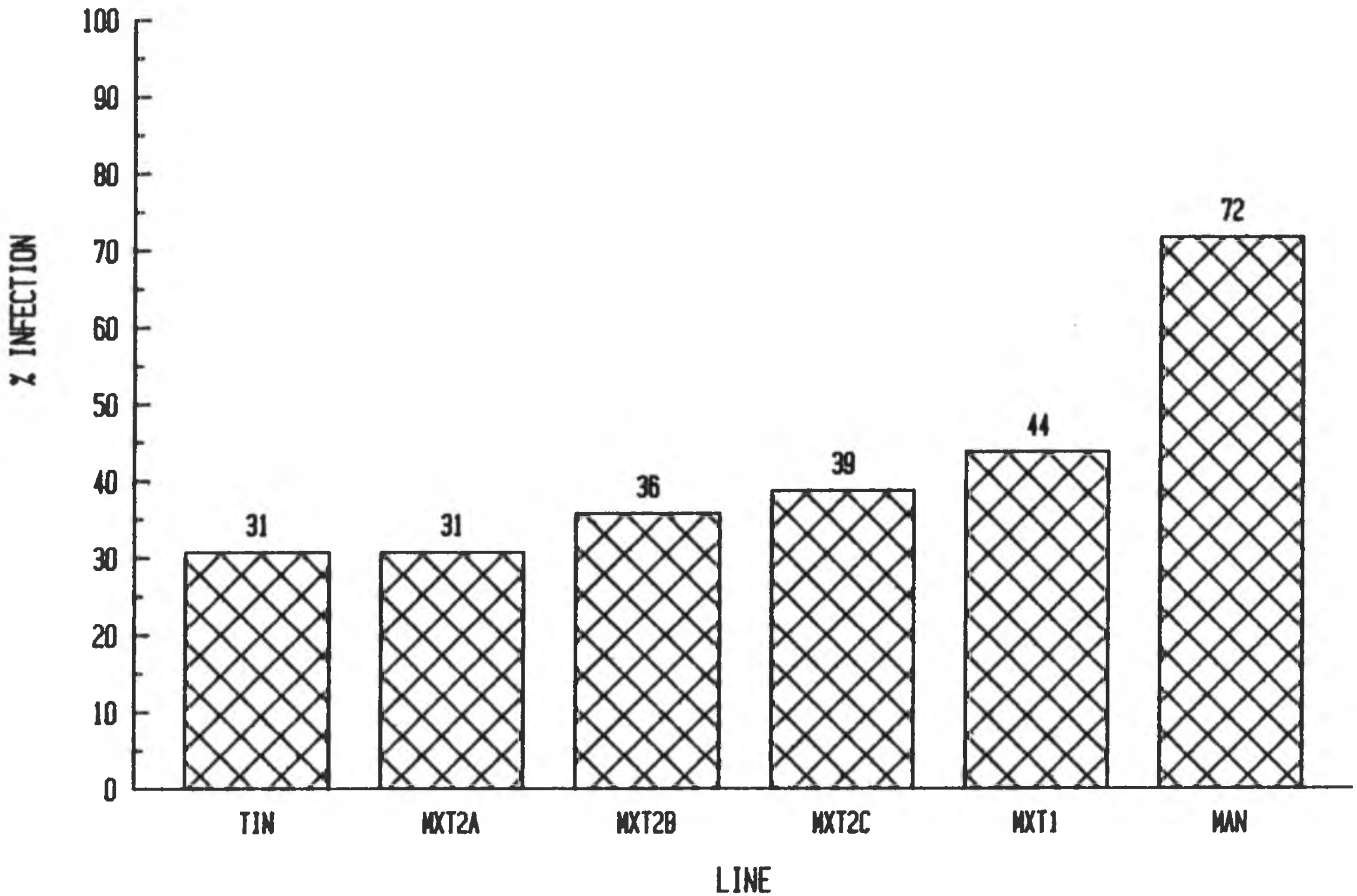
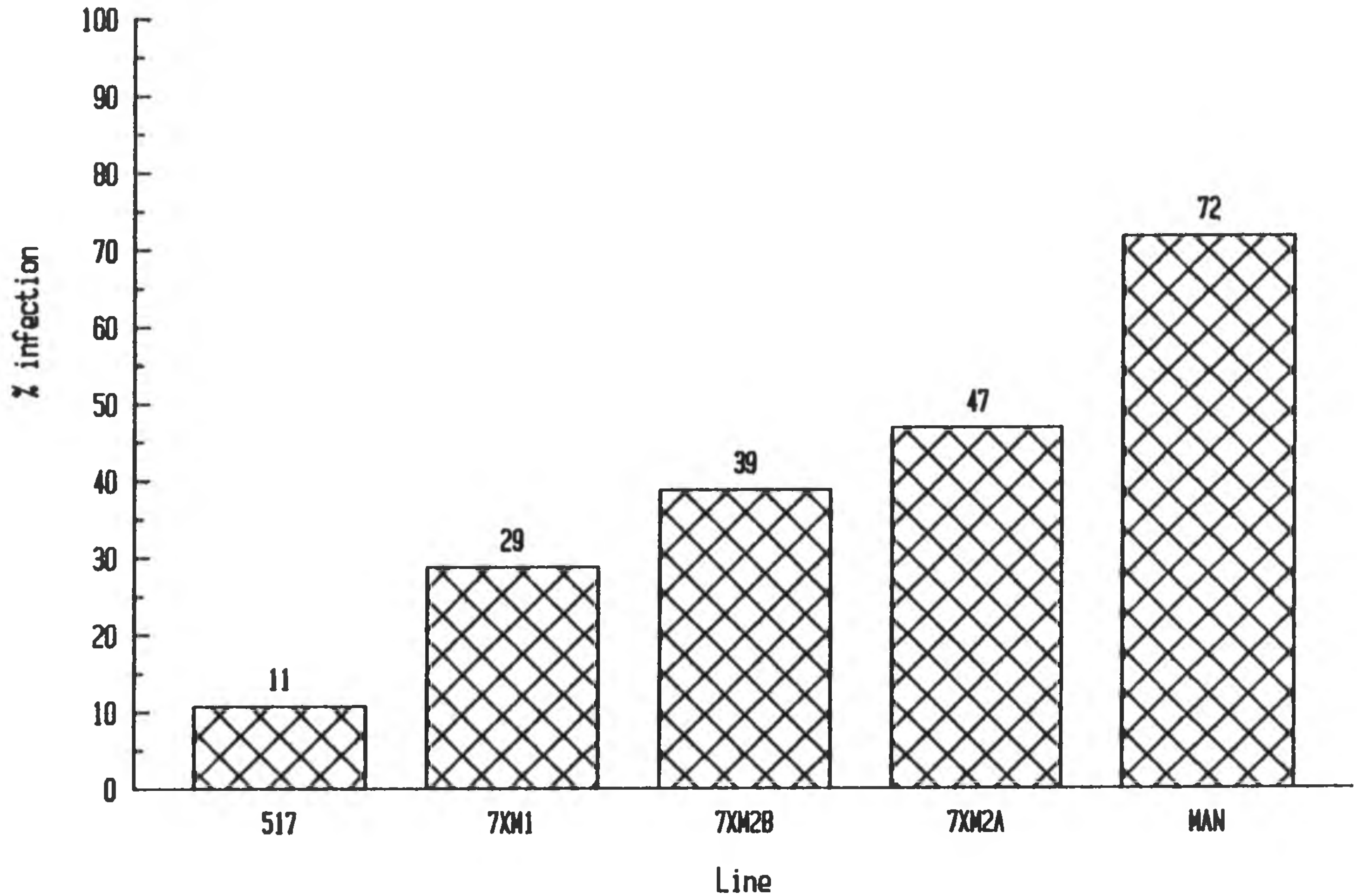


Figure 5. Obvious symptom method comparison of PI 342517, Manoa, and their progeny (Test 6).



When 'Tinto' and PI 342517 were crossed with 'Manoa', both F_1s were significantly different from 'Manoa' but not from the parents. They also were not significantly different from each other. The F_2s from these crosses differed somewhat in whether or not they were significantly different from the parents, probably due to variability in both the F_2s and the testing procedure. However, despite this, none of these F_2s were significantly different from each other. Thus, the contention that 'Tinto' and PI 342517 may very well have the same gene or genes for resistance remains viable.

The F_1 results from the Hawaii Kai trials (Test 2 and 4) support the hypothesis that the resistance in 'Tinto' and PI 342517 are the same and shows some dominance. When the ratings of these two tests were combined (Table 21), PI 342526, 'Manoa', PI 342510, 'Batavia', and PI 167128 were all significantly different from 'Tinto' and PI 342517 but not from each other. Therefore PI 342526, 'Manoa', PI 342510, 'Batavia', and PI 167128 were classified as susceptible and 'Tinto' and PI 342517 were classified as resistant. PI 342522 was not included in either class because it was not significantly different from the members of the susceptible class and PI 342517. Thus, using these class designations there was one cross of resistant x resistant (R x R) and five crosses of resistant x susceptible (R x S), making a total of four groups,

Table 21. Combined results of Kamilonui, Oahu field trials (Test 2 and 4).

Lettuce line or cross	# of plants rated	Mean[y]
PI 342517 X Batavia	4	1.75 A[x]
PI 342517 X Tinto[z]	18	1.83 AB
Tinto	24	1.88 AB
PI 342517	13	2.23 ABC
Batavia X Tinto	4	2.25 ABC
PI 342526 X Tinto	4	2.50 ABCD
PI 342517 X PI 342522[z]	15	2.53 ABCD
PI 342522 X PI 342526	12	2.75 ABCDE
PI 342517 X PI 342526	13	2.85 ABCDEF
PI 342522 X Tinto[z]	6	3.00 BCDEFG
PI 342522	17	3.12 CDEFG
Manoa X 342517	5	3.40 CDEFG
Manoa X PI 342526	6	3.50 DEFG
PI 342526	18	3.50 DEFG
Manoa	19	3.68 DEFG
Batavia X PI 342522	11	3.73 EFG
PI 342510	11	3.82 EFG
Batavia	12	4.00 FG
PI 167128	14	4.14 G

Z Crosses include reciprocals.
Y Disease rating 1-5, class 1 is most resistant, rated 7 weeks after transplanting at marketable maturity.
X Mean separation by Waller-Duncan multiple range test, 5% level.

resistant (R), susceptible (S), (R x R), and (R x S) (Table 22). A t test was used to judge differences between the four groups (Table 23).

As expected, there was a difference between (R) and (S) at the 1% level. (R x S) and (S) also showed a difference from each other at the 1% level. (R x S) and (R) showed a difference from each other at the 5% level but not at the 1% level. There was no difference between (R x R) and (R).

These results support the hypothesis that the resistance is inherited in an intermediate fashion with some dominance since the (R x S) group is significantly different from both the (S) group and the (R) group at the 5% level, but only from the (S) group at the 1% level. This could be explained by a multigene complex with at least one of the genes being dominant for resistance.

In (R X R) versus (R), both groups had some susceptible plants, but they showed no difference from each other at the 1% level. Therefore this test also supports the hypothesis that 'Tinto' and PI 342517 have the same gene or genes for resistance.

Table 22. Parents and F_1s classified as susceptible, resistant, susceptible x resistant, and resistant x resistant.

Line or cross	# of plants	Mean rating
PI 167128 (S)[z]	14	4.14
Batavia (S)	12	4.00
PI 342510 (S)	11	3.82
Manoa (S)	19	3.68
PI 342526 (S)	18	3.50
Total susceptible (S)	74	3.80
PI 342517 (R)	13	2.23
Tinto (R)	24	1.88
Total (R)	37	2.00
Manoa x PI 342517 (S x R)	5	3.40
PI 342517 x PI 342526 (S x R)	13	2.85
PI 342526 x Tinto (S x R)	4	2.50
Batavia x Tinto (S x R)	4	2.25
PI 342517 x Batavia (S x R)	4	1.75
Total (S x R)	30	2.67
PI 342517 x Tinto (R x R)	18	1.83
Total (R x R)	18	1.83

z Grouping symbols (S)=susceptible, (R)=resistant, (SxR) =susceptible x resistant, (RxR)=resistant x resistant.

Table 23. Comparison of four groups classified by Tomato Spotted Wilt Virus resistance.

Group[z]	Mean rating		Group	Mean rating
(S)	3.80 **[y]	----------------	(R)	2.00
(SxR)	2.67 *	----------------	(R)	2.00
(SxR)	2.67 **	----------------	(S)	3.80
(RxR)	1.83 ns	----------------	(R)	2.00

Z Grouping symbols (S)=susceptible, (R)=resistant, (SxR) =susceptible x resistant, (RxR)=resistant x resistant.
Y ** significance at 5% level, * significance at 1% level, ns non-significant in t test.

Summary and Conclusions

Nature of TSWV on Lettuce and Testing Procedures

TSWV is transmitted in nature by thrips. The first symptoms on lettuce plants can appear 2-3 weeks after initial thrips damage. Lettuce plants that develop symptoms before bolting generally do not survive. TSWV infections can also be instigated by mechanical inoculation as described previously in the inoculation section. Symptoms first appear 1-3 weeks after inoculation. However, there was a high degree of variability for rate of infection within lines and between weeks with the method used.

For a more precise determination of TSWV resistance inheritance in lettuce, testing methods which produce consistent results from week to week are needed. Since climatic conditions, thrips, and host plant condition were observed as causing differences between greenhouses, these factors should be standardized. The use of growth chambers should alleviate the climatic and host condition factors, while the use of laboratory raised thrips could be used for inoculation purposes. In addition, field testing in a TSWV infected area should also be used. By field testing, resistance can be evaluated under the normal climatic conditions and level of infective thrips population found in the field.

Interpretations of Resistance

'Tinto' and PI 342517 are significantly more resistant from TSWV than the susceptible commercial variety 'Manoa' (Green Mignonette) at 3 weeks after mechanical inoculation, they are also significantly more resistant under conditions of natural infection in the field 7 weeks after being transplanted.

Since no difference was found between 'Tinto' and PI 342517, the resistance in them could be controlled by the same gene or genes. This resistance showed over the course of testing to be either dominant or partially dominant in F_1 populations. Because of the variability of infection rates from week to week it was impossible to determine with any precision the number of genes governing this resistance. Therefore, the resistance in 'Tinto' and PI 342517 can be best described at this time as quantitative inheritance controlled by dominant to partially dominant gene or genes. This resistance can be broken down under conditions of high disease pressure such as when plants are reinoculated one week after inoculation.

Applications for Resistance

The resistance in 'Tinto' and PI 342517 is obviously not immunity since some plants from both these lines developed systemic symptoms. It would seem more plausible that resistance is due to resistance to virus inoculation. Bjorling (1966) describes this sort of resistance as the ability of some varieties when exposed to equal chances of infection with other varieties to be less likely to become infected.

Additional testing is needed to determine if this type of resistance is of a level worth incorporating into commercial lettuce varieties. Should further testing show that it is worthwhile, crosses between the resistant and susceptible parents would have to be grown out for several generations to restore the homozygosity of the commercial lettuce varieties.

LITERATURE CITED

Abu Bakar, A. K. 1974. Studies on isolates of TSWV in Hawaii. M.S. Thesis. University of Hawaii. 1-56.

Bailey, L. H. 1976. Hortus Third. Macmillan Publishing Company. 631.

Bald, J. G., and G. Samuel. 1931. Investigations on spotted wilt of tomatoes. Australian Coun. Sci. Ind. Res. Org. Bull. 54.

Best, R. J. 1968. Tomato spotted wilt virus. In Smith K. M., and M. A. Lauffer (eds). Advances in Virus Research. New York Academic Press. 65-146.

Best, R. J. and H. P. Gallus. 1953a. Strains of tomato spotted wilt virus. Aust. J. Sci. 15: 212-214.

Bjorling, K. 1966. Virus resistance problems in plant breeding. Acta Agriculturae Scandinavica Suppl. 16. 120-132.

Boelma, B. H., and M. Bolton 1984. Diseases and pests in lettuce. Bull. of Hort. Res. Institute, Pretoria 1-4.

Borchers, E. A. 1957. Some factors concerning the nature of resistance of the mingonette variety of lettuce to the spotted wilt virus. Ph.D. Dissertation. University of California, Davis. 1-30.

Bos, L. 1981. Wild plants in the ecology of virus diseases. In Maramorosch, K. and K. F. Harris (eds). Plant Diseases and Vectors: Ecology and Epidemiology. New York Academic Press. 1-34.

Brittlebank, C. C. 1919. Tomato diseases. J. Agr. Victoria 17: 213-235.

Cho, J. J., R. F. L. Mau, W. C. Mitchell, D. Gonsalves, and L. S. Yudin. 1986. Host list of TSWV susceptible plants. Submitted for publication.

Cho, J. J., R. F. L. Mau, D. Gonsalves, and W. C. Mitchell. 1986. Resevoir weed hosts of TSWV. Plant Disease 70: 1014-1017.

Docampo, D. and F. Nome. 1970. TSWV in the cultivation of lettuce in the provinces of Aconcagua and Valparaiso. Chile Estac. Exp. Agron. Bol. Tec. 31: 24-28.

Duffus, J. E. 1971. Role of weeds in the incidence of virus diseases. Ann. Rev. Phytopath. 9: 319-340.

Fichter, G. S. 1966. Insect Pests. Golden Press. 72,89.

Finlay, K. W. 1953. Inheritance of spotted wilt resistance in the tomato. II. Five genes controlling spotted wilt resistance in four tomato types. Aust. J. Biol. Sci. 6: 153-163.

Francki, R. I., and T. Hatta. 1981. Tomato spotted wilt virus. In Kurstak, E. (ed). Handbook of Plant Virus Infections and Comparative Diagnosis. American Elsevier Publishing Company Incorporated. 491-512.

Harris, M. R. 1939. A survey of the spotted wilt disease of lettuce in the Salinas Valley. Bull. of the Dept of Agr. State of California. 28: 201-213.

Holmes, F. O. 1948b. Resistance to spotted wilt in tomato. Phytopathology. 38: 467-473.

Ie, T. S. 1970. TSWV. Commonwealth Mycological Institute / Assoc. Appl. Biologist. Descriptions of Plant Virus 39: 1-4.

Illingworth, J. F. 1931. Yellow spot of pineapple in Hawaii. Phytopathology. 21: 865-880.

Kikuta, K., and W. A. Frazier. 1946. Breeding tomatoes for resistance to spotted wilt in Hawaii. Proc. Amer. Soc. Hort. Sci. 47: 271-276.

Kikuta, K., J. W. Hendrix, and W. A. Frazier. 1945. Pearl Harbor, a tomato variety resistant to spotted wilt in Hawaii. Hawaii Agr. Exp. Sta. Circ. 24: 1-4.

Lindkvist, K. 1960b. Inheritance studies in lettuce. Hereditas. 46: 387-470.

Linford, M. B. 1932. Transmission of the pineapple yellow-spot virus by Thrips tabaci. Phytopathology. 22: 301-324.

Magee, C. J. 1934. Spotted wilt of lettuce. Agr. Gas. New South Wales. 47: 99-100,118.

Mau, R. F. L. 1986. Tomato spotted wilt virus control. Leafy Vegetables Industry Analysis. 3: 23-30.

Moller, W. J., and I. S. Rogers. 1960. Spotted wilt of lettuce. J. Dept. Agr. South Australia. 64: 94-95,106.

Norris, D. O. 1946. The strain complex and symptom variability of tomato spotted wilt virus. C.S.I.R.O. (Australian) Bull. No. 202.

Osborn, T. G. B. 1919. Two serious new wilt diseases. J. Agr. South Australia. 23: 437.

Parris, G. K. 1938. A new virus Disease of tomatoes for Hawaii. Hawaii Agr. Expt. Stat. Rept. : 41-42.

Parris, G. K. 1940. Mechanical transmission of yellow-spot virus: Evidence for identity with spotted wilt virus. Phytopathology. 30: 299-312.

Pittman, H. A. 1927. Spotted wilt of tomatoes. J. Australian Coun. Sci. Indust. Res. 1: 74-77.

Ryder, E. J. 1979. Lettuce. Leafy Salad Vegetables. A.V.I. Publishing Company Incorporated. 13-94.

Ryder, E. J. 1986. Lettuce breeding. In Bassett, M. J. (ed). Breeding Vegetable Crops. A.V.I. Publishing Company Incorporated. 436-476.

Ryder, E. J. and A. S. Johnson. 1974b. Mist depollination of lettuce flowers. HortScience. 9: 584.

Sakimura, K. 1940. Evidence for the identity of the yellow-spot virus with the spotted-wilt virus: Experiments with the vector, *Thrips tabaci*. Phytopathology. 30: 281-299.

Sakimura, K. 1961a. Techniques for handling thrips in transmission experiments with the tomato spotted wilt virus. Plant Dis. Rept. 45: 766-771.

Samuel, G., J. G. Bald, and H. A. Pittman. 1930. Investigations on "spotted wilt" of tomatoes. Australian Coun. Sci. Indust. Res. Bull. 44.

Smith, K. M. 1931. Studies on potato virus diseases. VIII. On a ringspot virus affecting solanaceous plants. Ann. Appl. Biol. 18: 1-15.

Smith, K. M. 1932. Studies on plant virus diseases. XI. Further experiments with a ringspot virus: Its identification with spotted wilt of the tomato. Ann. Appl. Biol. 19: 305-330.

Statistics of Hawaiian Agriculture. 1985. 1-100.

Tompkins, C. M., and M. W. Gardner. 1934. Spotted wilt of head lettuce (abstr.). Phytopathology. 24: 1135-1136.

Upreti, G. 1983. Inheritance of resistance to tomato spotted wilt virus in tomatoes. M.S. Thesis. University of Hawaii. 1-89.

Upreti, G. 1984. Inheritance of resistance to tomato spotted wilt virus in tomatoes. I.A.A.S. Nepal J. 4: 19-25.